The University of Chicago

THE ESSENTIAL ROLE OF THE ADRENAL CORTEX IN THE HYPERTROPHY OF THE OVOTESTIS FOLLOWING OVARIECTOMY IN THE HEN

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
SEPTEMBER, 1942

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WILLIAM FRANCIS HEWITT, JR.

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It is well known that the adrenals are associated with sex function in the human and in lower animals. Several sexually active substances have been isolated from the adrenal cortex: theelin (1), progesterone (2), and androsterone, desoxycorticosterone and other steroids (3, 4).

Functional interrelationship between the adrenal cortex and the ovary or testis has been repeatedly indicated. Moon (5) obtained vaginal introitus and signs of estrus in spayed rats injected with adrenocorticotropic hormone. Casida and Hellbaum (6) were able to stimulate follicle maturation, ovulation, and luteinization in rats 21 to 25 days old by injection of adrenal extracts. Bülbring (7) noted that in the adrenalectomized drake the normal enlargement of the testis from January to June could be maintained only by increased administration of adrenocortical extracts. Freed, Brownfield, and Evans (8) found testicular degeneration in the rat following adrenalectomy, and concluded that this effect was not mediated by the pituitary, since injections of pregnancy urine restored accessory sex organs but not gonads. Herrick and Torstveit (9) adrenalectomized cocks, producing a capon-like bird showing testicular degeneration. Herrick and Finerty (10) believed the pituitary to be intermediary in this effect, since the "castrate cells" typical of the capon's hypophysis were not observed in the adrenalectomized bird, whose pituitary instead showed a basophilic degeneration.

The adrenal cortex has been shown also to influence accessory sex organs. Davidson and Moon (11) found that adrenocorticotropic hormone produced in the castrate male rat an active condition of the prostatic epithelium, simultaneous with adrenocortical hypertrophy. Hodler (12) in the guinea pig and Davidson (13) in the rat did not observe this condition if the adrenals were absent. Burrill and Greene (14) observed that the ventral prostate of the immature male castrate rat was maintained in the presence

of the adrenal, but suffered complete and rapid degeneration if the adrenals were removed.

The present investigation was stimulated by comparing the results of Herrick and co-workers (op. cit.) with those of Domm (15, 16, 17). It was the latter series of experiments which clearly established the nature of the changes in the right rudimentary gonad of the hen after removal of the left (normally developed) ovary: the right gonad (ovotestis) enlarges, changes to a testis-like organ, and seems to secrete male sex hormone. This change in the ovotestis after ovariectomy is presumably under some sort of masculinizing influence; in the light of the established sexual possibilities of the adrenal cortex, it was believed that this tissue might play a part. To investigate this hypothesis, it was determined first to ascertain whether or not the adrenal was necessary to the hypertrophy of the ovotestis following ovariectomy.

Methods and Materials

Sixty-five White Leghorn chickens of both sexes are included in the results. About thirty-five others were lost by disease or operative hemorrhage, or were not included because of failure to make some essential observation. Some animals were obtained from a poultry farm at the age of approximately three months; most, however, were bought on the open market at about one and one-half years of age. Birds were kept in individual cages and fed chopped scratch feed supplemented by lettuce and cabbage; 0.9 per cent saline was given for drinking water. The period of observation in most cases was two to three months; in a few instances this was extended to six or eight months.

Organs were destroyed in situ by electrocautery. Birds were injected intramuscularly with 5 mgms. of desoxycorticosterone acetate (in oil) 24 to 48 hours before operation, immediately upon completion of the operation, 24 hours postoperatively, and 48 to 72 hours after operation. Nearly complete aseptic precautions were taken after one or two deaths from sepsis were observed. In adult hens ovariectomy was difficult and often fatal, because of the size and vascularity of the organ. Anesthesia was induced by nembutal (30 mgms. per kilogram body weight) given intraperitoneally. Sham operation consisted of opening the body cavity at the usual site, keeping it open for the length of time required for the average ovariectomy (about 30 minutes), cauterizing a moderate

amount of fat and connective tissue in the region of ovary or adrenal, and closing up.

To avoid possible effects of hemorrhage on the size of secondary characters, animals were sacrificed by overdosage of nembutal. Organs were fixed in Bouin's solution, sectioned at 8 to 12 mu (usually at 10), stained with hematoxylin and eosin and mounted in balsam. A thorough search was made for adrenocortical rests; suspicious nodules were examined microscopically.

Combs, wattles, earlobes and spurs were measured no less often than once per month (usually every two weeks); dimensions recorded were of the two longest axes at right angles to one another.

Since the observations of Benoit (18) and others have shown an important influence of light upon gonads, an attempt was made to control this factor: heavy cloth was nailed over windows, and lights were on only from 7:30 A.M. to 5:00 P.M. No seasonal variations in size of primary or secondary sexual characters; it is therefore believed that light-induced differences were minimized.

Results

Secondary characters.--Data are summarized in Table 1. Earlobe and wattle changes paralleled those of the comb, and are not given in order to conserve space. "Before" means at time of first operation; "after" means at death or sacrifice of the animal.

The mature normal males used as controls showed no change in secondary characters. The bilaterally adrenalectomized, unilaterally adrenalectomized, and bilaterally orchidectomized groups, however (with one individual exception) showed significant diminution in comb size. These results confirm those of Torstveit and Herrick (op. cit.), indicating an influence of the adrenal cortex upon secondary characters (perhaps through the hypophysis).

The bilaterally adrenalectomized and ovariectomized-adrenalectomized hens showed sharp and consistent decreases in comb size, indicating the same role for the adrenal cortex in the female as in the male. The change in the animals which died unoperated was consistent, but so small as to indicate that cachexia was not responsible.

Changes in the other groups of animals are not considered significant.

TABLE 1

COMBS, LENGTH PLUS HEIGHT (AVERAGES), CENTIMETERS

Number	Group	Comb		Number Showing		
		Before	After	Decrease	Increase	No Change
4	Normal.....	17.25	17.25	0 (0.0)	0 (0.0)	4
1	Unilaterally orchidectomized...	10.5	10.4	1 (0.1)	0 (0.0)	0
1	Bilaterally orchidectomized...	19.0	17.0	1 (2.0)	0 (0.0)	0
5	Unilaterally adrenalectomized..	16.7	14.8	4 (3.5)	0 (0.0)	1
3	Bilaterally adrenalectomized..	16.0	13.2	2 (6.9)	1 (0.5)	0
	Females					
11	Normal.....	9.9	11.4	2 (1.8)	4 (6.8)	5
5	Sham control.....	11.1	12.5	3 (2.5)	2 (8.0)	0
4	Subtotal ovarian damage.....	10.7	12.6	1 (0.8)	1 (8.5)	2
8	Ovariectomized....	11.8	12.4	4 (2.7)	4 (8.5)	0
8	Unilaterally adrenalectomized..	10.3	10.2	4 (5.0)	3 (3.0)	1
5	Bilaterally adrenalectomized..	12.5	8.5	4 (5.0)	0 (0.0)	1
3	Died unoperated...	9.8	9.5	3 (0.4)	0 (0.0)	1
7	Ovariectomized and bilaterally adrenalectomized..	11.7	8.6	7 (5.0)	0 (0.0)	0

Testis.--Results are summarized in Table 2. Testis size did not change during the period of observation, in normal male controls. Unilateral adrenalectomy had only a moderate effect; since the change was consistent, however, it is believed to be significant. This adds to the observations of Herrick and Torstveit on the bilaterally adrenalectomized cock. As reported by these workers, the birds in the present experiment suffered a profound and consistent diminution in testis size. Upon microscopic examination such testes showed degeneration, slight in the unilaterally and nearly complete in the bilaterally adrenalectomized

animals.

TABLE 2

TESTIS SIZE, LENGTH PLUS WIDTH, CENTIMETERS

Bird Number	Group	Testis	
		Before	After
32	Normal.....	5.0	5.0
38	Normal.....	8.0	8.0
	Mean.....	6.5	6.5
29	Unilaterally adrenalectomized....	7.5	6.0
31	Unilaterally adrenalectomized....	5.5	3.1
33	Unilaterally adrenalectomized....	8.0	6.2
41	Unilaterally adrenalectomized....	8.5	6.2
43	Unilaterally adrenalectomized....	4.5	3.8
	Mean.....	6.8	5.1
37	Bilaterally adrenalectomized.....	7.5	5.0
44	Bilaterally adrenalectomized.....	8.5	2.3
81	Bilaterally adrenalectomized.....	8.0	3.5
	Mean.....	8.0	3.6

Ovotestis.--The symbol "---" in Table 3 indicates that the ovotestis was difficult to find, and presented the normal rudimentary appearance. Dimensions in such cases were so small as to be meaningless. Hypertrophy of the ovotestis was observed in the ovariectomized group, and in one of the four birds suffering subtotal ovarian damage. On autopsy this latter hypertrophied organ measured 0.8 cms. (combined length and width). In no single instance did the ovotestis of an adrenalectomized-ovariectomized bird undergo hypertrophy. This is taken to indicate the essential role of the adrenal cortex in such growth of the rudimentary right gonad.

Saddle-feathering, growth of spurs, voice changes, and aggressive behavior were not invariably associated with an hypertrophied ovotestis. Bird 49, for example (Table 3), which showed no hypertrophy at autopsy, exhibited, nevertheless, the usual outward male characteristics. Bird 70, showing hypertrophy, suffered, if anything, a slight decrease in secondary characters.

Hypertrophied ovotestes presented a testis-like appearance microscopically, as was expected from the work of Domm and others.

TABLE 3

OVOTESTIS, LENGTH PLUS WIDTH, CENTIMETERS

Bird Number	Group	Ovotestis	
		Before	After
47	Ovariectomized.....	---	0.5
49	Ovariectomized.....	---	---
70	Ovariectomized.....	---	2.0
75	Ovariectomized.....	---	0.7
89	Ovariectomized.....	---	---
91	Ovariectomized.....	---	0.8
95	Ovariectomized.....	---	2.0
100	Ovariectomized.....	---	0.5
	Mean.....	---	1.1*
17	Ovariectomized and bilaterally adrenalectomized.....	---	---
19	Ovariectomized and bilaterally adrenalectomized.....	---	---
21	Ovariectomized and bilaterally adrenalectomized.....	---	---
63	Ovariectomized and bilaterally adrenalectomized.....	---	---
76	Ovariectomized and bilaterally adrenalectomized.....	---	---
82	Ovariectomized and bilaterally adrenalectomized.....	---	---
84	Ovariectomized and bilaterally adrenalectomized.....	---	---
88	Ovariectomized and bilaterally adrenalectomized.....	---	---
	Mean.....	---	---

*Includes only those birds showing a hypertrophy.

General comment.--The shortness of the period of observation probably decreased the uniformity and consistency of dimensions of gonads and secondary characters, but was unavoidable because of lack of space. Those kept six to eight months showed more pronounced, more uniform, and more consistent changes. It is believed that future work over a longer period will increase the significance of the data.

Another observation of Torstveit and Herrick confirmed in the present study is the resistance of the fowl to disturbances of salt-and-water metabolism: although many birds died from what

was judged to be adrenal insufficiency, the mortality by no means approached that of the mammal. Even those birds who at autopsy showed no adrenal tissue seemed to suffer no ill effects when on tapwater for 48 hours as a test for adrenal insufficiency. Torstveit and Herrick state that their birds seemed in perfect health until a few hours before death, which came swiftly in collapse; this same type of death was observed consistently in the present study. Occasionally, however, a unilaterally adrenalectomized bird died, seemingly from adrenal insufficiency: saline-thirst and asthenia were evident, and autopsy ruled out sepsis, hemorrhage, pneumothorax, and pneumonia. Possibly in these animals the remaining adrenal did not begin compensatory hyperfunction soon enough.

An apparent influence of adrenal upon ovary is suggested by the small, immature-appearing ovaries in the adult after unilateral or bilateral adrenalectomy.

In some bilaterally adrenalectomized birds "moulting" occurred--the loss of long, heavily quilled feathers in great numbers. This was not observed in any other group of birds. Possibly such feathering is another secondary character subject to adrenocortical influence.

Discussion

The principal significance of these results is the indication of adrenocortical gonadotropism--in the absence of neoplasm or other pathology of the gland, and without administration of excessive quantities of adrenocortical substance. Is this influence a direct one, or is the pituitary an intermediary? Conflicting reports have been cited--those of Freed, Brownfield, and Evans opposing those of Herrick and co-workers. In order to clear up this point further work is necessary, on the hypophysectomized bird and with adrenocortical substances.

Another indication of these experiments is the apparent unreliability of comb, wattle, or earlobe dimensions as an index to any but maximum derangements in the internal endocrine environment. It is not the intent of this paper to ascribe such unreliability to the capon-comb method for androgen assay. It is rather desired to point out a difference, under the conditions of the present work, between the variation in dimensions and the quantitation possible by direct inunction of substances upon the comb. In an investigation such as the present one, dimensions of second-

ary characters seem to have at most a secondary, or qualitative, significance.

It seems worth mentioning that the fowl is apparently well suited to endocrine research: (1) Secondary characters furnish easily observed indications of inner changes without the necessity of biopsy or autopsy. (2) The ovotestis is a good qualitative index of gonadotropic changes in the organism. (3) The fowl's seeming relative resistance to the effects of adrenal insufficiency aids the study of adrenocortical factors.

Summary

The work of Herrick and Torstveit is confirmed: testis atrophy and an external capon-like appearance follow bilateral adrenalectomy in the White Leghorn cock. It is further established that in the hen the presence of the adrenal is necessary to the conversion of the right rudimentary gonad into a testis-like organ following ovariectomy. The usual hypertrophy of the ovotestis was observed in ovariectomized birds, but not in hens which had been adrenalectomized as well as castrated. Measurements of comb, wattles, and earlobes were found, under the conditions of these experiments, to yield only qualitatively significant results. The necessity for further work is indicated.

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